An Alternative Possible Origin of the A2 Mating Type of *Phytophthora infestans* Outside Mexico

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ABSTRACT

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Progeny derived from selfed oospores of an A1 (isolate 533) or A2 (isolate 550) field isolate of *Phytophthora infestans* induced by mating-type-specific hormones consisted of both the A1 and A2 mating types. Oospore progeny produced by selfing of the hybrid of these field isolates also contained the self-fertile A1A2 type in addition to the A1 and A2 types. Selfed-oospore progeny produced by six A1 cultures in S_1 of isolates 533 and 550 consisted of the A1, A2, and A1A2 types; of the A1 and A2 types; or of the A1 type only. One of the selfed progeny produced by A2 cultures in S_1 of isolate 533 consisted of the A1 and A2 types, while the other consisted of the A2 type only. Results from this study suggest that some individuals of the A2 mating type of *P. infestans* existing outside Mexico may have originated from selfed oospores produced by the A1 mating type after arriving at the present host countries.

Gallegly and Galindo (13) reported in 1958 the discovery of oospores produced by *Phytophthora infestans* (Mont.) de Bary in nature in Mexico and the detection of an approximately equal ratio of A1 and A2 mating types of this fungus among 95 Mexican isolates collected in different areas from 1952 through 1956. However, the 105 isolates recovered by Smoot et al (33) from the United States, Canada, western Europe, South Africa, and the West Indies were all of the A1 mating type. Thereafter, no study on this subject was documented in western countries until 1984, when interest was revived (9,21,22,26,30,34) by Hohl and Iselin's report (14) of the isolation of both the A1 and A2 mating types of *P. infestans* in Switzerland. These events led to the recent suggestion by Fry et al (11) that the A2 mating type of *P. infestans* was confined to Mexico, whereas the A1 mating type was distributed worldwide from the 1950s to the late 1970s, and that the recent worldwide population changes of this pathogen almost certainly resulted from migration.

Smoot et al (33) suggested in 1958 that the A1 mating type of *P. infestans* probably was predominant in the areas where they obtained their isolates. This does not necessarily indicate the sole existence or even the predominance of the A1 mating type in other areas of the same countries in 1958 or since then. The coincidental isolation of A2 of *P. infestans* for the first time in 1981 in Switzerland, Wales, and England led Shaw (29) to suggest that A2 probably has been present in western Europe for many years, at least since 1981. The events mentioned above indicate that besides Mexico and those areas outside Mexico surveyed by Smoot et al (33), the distribution of mating types of *P. infestans* worldwide remained mostly unknown until after 1984.

Fyfe and Shaw (12) reported that single-sporangium and single-hyphal-tip progeny derived from self-fertile isolates of *P. infestans* from England, Wales, and Egypt consisted of A1 and A2 cultures

and a low frequency of self-fertile cultures. This suggests the existence of both mating types of *P. infestans* in the United States (8) and Ireland (23) in the 1910s and in Australia, England, and Holland (2) in the 1920s because self-fertile isolates of *P. infestans* were recovered from these countries during those periods. Since then, self-fertile isolates of *P. infestans* also have been recovered outside Mexico (12,35).

Both mating types of *P. infestans* were also found in Japan in the 1930s. In 1937, Hori (15) recovered nine isolates of *P. infestans* (six from potato and three from tomato) from diseased tissues collected from 20 locations surveyed throughout Japan. The potato isolates, which produced few oospores in oogonia without discernable antheridia in single cultures, were designated (−) strains, while tomato isolates, which did not produce oospores in single cultures, were designated (+) strains. When (−) and (+) strains were paired on the same medium, numerous oospores in oogonia with amphygenous antheridium were produced (16). In addition to the ability to produce oospores in single cultures, the (−) strains also grew on oatmeal agar. These characteristics coincide with those of A2 mating type cultures of *P. infestans* recently found in Japan (17,22). The (+) strains reported by Hori and Yoshida (16) also coincide with the recently isolated A1 mating type in lack of ability to produce oospores in single cultures (22).

It is conceivable that both the A1 and A2 mating types of *P. infestans* have coexisted in a number of countries throughout the world since the beginning of the 20th century. This may be used to explain the detection of both mating types in most countries surveyed since the resumption of research on this subject in 1984 (9,10,14,21,22,27,34).

In an area where the A1 mating type of *P. infestans* arrived first, the later appearance of the A2 mating type of the same species may result from migration (11) or from mating type change from the original A1 to A2 induced by aging (19) or exposure to various fungicides (1,4,19,20). I report here another possible origin of the A2 mating type of *P. infestans*.

MATERIALS AND METHODS

Fungi. Mexican isolates 533 (A1) and 550 (A2) of *P. infestans* were obtained from W. F. Fry. Isolate 930 (A1) is a hybrid from the cross between isolates 533 and 550 (6). Each isolate was derived from a single zoospore and maintained on V8 rye agar modified from the rye agar described by Caten and Jinks (3). Rye broth was obtained by soaking 50 g of whole rye grains in 1,100 ml of distilled water at 24 C for 24–36 h, followed by autoclaving for 30 min. The supernatant was filtered through four layers of cheesecloth and adjusted to 1,000 ml with distilled water. V8 rye agar was prepared by adding 5% V8 juice, 0.02% $CaCO_3$ and 2% Bacto agar to rye broth.

Formation of selfed oospores. Selfed oospores were obtained with the polycarbonate membrane method (18). A culture block (15 × 10 × 3 mm) of a 4-day-old A1 or A2 mating type was covered with a sterile polycarbonate membrane (0.2 μm, 90 mm in diameter; Nuclepore Co., Pleasanton, CA) in a petri dish and hormonally induced to produce oospores by a 4-day-old culture block of the opposite mating type placed on top. After incubation for 10 days at 19 C in darkness in a moist chamber, the membrane and the hormone producer on the top were removed and the culture block containing oospores on the bottom was further incubated for 10 days under the same conditions to obtain mature oospores.

Germination of oospores. The method of Chang and Ko (5) was used to induce oospore germination of *P. infestans*. An oospore suspension was obtained by grinding a culture block containing oospores with 50 ml of distilled water in an Omni mixer (DuPont Instruments, Newtown, CT) at 4,500 rpm for 1 min. The suspension was filtered successively through a 53-μm and a 20-μm sieve. Oospores retained on the 20-μm sieve were washed with tap water and resuspended in 10 ml of sterile distilled water. The oospore suspension was mixed with an equal volume of freshly prepared $KMnO_4$ solution at 0.5% (w/v). After the mixture was agitated for 15 min on a shaker, oospores were washed free of $KMnO_4$ on a 20-μm sieve with tap water. About 100–200 oospores were spread on S+L medium (24) amended with 0.01% asparagine and 2% Bacto agar. After autoclaving, this medium was supplemented with 100 mg of ampicillin, 50 mg of nystatin, and 10 mg of pentachloronitrobenzene per milliliter to prevent growth of contaminants. After incubation at 19 C under continuous cool-white fluorescent light (2,000 lx) for 10–20 days, germinating oospores were individually transferred to V8 rye agar.

Determination of mating type. The mating type of each single-oospore culture of *P. infestans* was determined by pairing a small piece (approximately 3 × 3 × 3 mm) of culture with the same size culture of the A1 tester (isolate 533) or the A2 tester (isolate 550) on a V8 rye agar block (approximately 15 × 10 × 3 mm) in a petri dish. Ten blocks were placed in a petri dish at equal distance along the edge. After incubation at 19 C in darkness for 8–10 days, agar blocks were examined microscopically. Those isolates forming oospores when paired with the A2 tester were designated as being A1. Similarly, those forming oospores when paired with the A1 tester were A2 and those forming oospores with both the A1 and A2 testers were A1A2, which also formed oospores in the absence of the testers.

RESULTS

Progeny from selfing of wild types and a hybrid. Among the 92 selfed-oospore progeny of A1 isolate 533 of *P. infestans* obtained, 90 were of the parental A1 type and two became the A2 type (Table 1). In addition to the parental A2 type, selfed-oospore progeny of A2 isolate 550 also contained the A1 type. Progeny from selfing of the A1 hybrid 930 also consisted of both the A1 and A2 types. Moreover, there were three self-fertile cultures (A1A2) among the 180 progeny tested.

Progeny from selfing of S_1 of wild types. Among five A1 isolates in S_1 of isolate 533 tested, three produced selfed-oospore progeny consisting of both the A1 and A2 types (Table 2). Oospore progeny produced by isolate 908 also contained two of the A1A2 type. All oospore cultures derived from the other two A1 isolates were of the parental A1 type.

TABLE 1. Mating type distribution in selfed-oospore progeny of isolates 533, 550, and 930 of *Phytophthora infestans*

Parental isolate	Mating type	No. of single-oospore cultures[a]			
		Total scored	Mating type		
			A1	A2	A1A2
533	A1	92	90	2	0
550	A2	50	3	47	0
930[b]	A1	180	168	9	3

[a] Oospores were obtained by hormonal stimulation using the polycarbonate membrane method (18).
[b] Isolate 930 is a hybrid from the cross between isolates 533 and 550.

TABLE 2. Mating type distribution in single-oospore cultures derived from the selfing of S_1 progeny of isolates 533 and 550 of *Phytophthora infestans*

Parental isolate[a]	Mating type	No. of single-oospore cultures[b]			
		Total scored	Mating type		
			A1	A2	A1A2
908	A1	82	75	5	2
925	A1	40	40	0	0
926	A2	45	2	43	0
928	A1	40	35	5	0
932	A1	40	37	3	0
936	A1	43	43	0	0
948	A2	41	0	41	0
912	A1	48	47	0	1

[a] All isolates are S_1 of isolate 533 (A1) except isolate 912, which is S_1 of isolate 550 (A2).
[b] Oospores were obtained by hormonal stimulation using the polycarbonate membrane method (18).

One of the two A2 isolates in S_1 of isolate 533 tested produced oospore progeny consisting of both the A1 and A2 types. However, all the 41 oospore progeny produced by the other A2 isolate were of the parental A2 type.

The only A1 isolate 912 in S_1 of isolate 550 tested produced oospore progeny containing only the A1 type. Although no A2 type was produced, one of the 48 oospore cultures obtained was of the A1A2 type (Table 2).

DISCUSSION

Results from this study show that five of eight A1 isolates of *P. infestans* tested can produce A2 offspring amounting to 2–13% of each selfed progeny. The A1 mating type of *P. infestans* can produce selfed oospores in response to stimulation by the A2 mating type of *P. capsici* (31), *P. drechsleri* (28,32), *P. palmivora* (31), or *P. parasitica* (1,7) in addition to *P. infestans* (6,28,31). Therefore, some of those A2 mating types of *P. infestans* existing outside Mexico may have originated from selfed oospores produced by the A1 mating type.

Two of the three A2 isolates of *P. infestans* tested also produced A1 offspring amounting to 4–6% of each selfed progeny. Since the A2 mating type of *P. infestans* has been shown to produce selfed oospores in response to stimulation by the A1 mating type of *P. infestans* (6) or *P. parasitica* (7), and since certain A2 isolates can produce selfed oospores in single cultures (22), some of those A1 mating type existing in nature also may have originated from oospores produced by selfing of the A2 mating type.

The results of this study show that an A2 type (isolate 926) that was derived from an A1 type (isolate 533) by selfing can again produce A1 type selfed-oospore cultures. This clearly demonstrated the reversibility of the mating type change in *P. infestans*. A similar phenomenon has been observed in *P. parasitica* (19,20) and *P. cinnamomi* (1). These observations do not support the hypothesis proposed by Sansome (25) that A1 is homozygous recessive *aa* and A2 is heterozygous *Aa* because the homozygous recessive genotype should not segregate.

Three of the eight A1 isolate of *P. infestans* tested produced the self-fertile (A1A2) type amounting to about 2% of each selfed progeny. Since asexual progeny of self-fertile isolates of *P. infestans* consisted of both the A1 and A2 mating types (12), the A2 offspring produced by self-fertile isolates derived from selfed-oospore cultures of the A1 mating type may also contribute to the A2 mating type population of *P. infestans* in nature. It is also possible that the unstable self-fertile type existing in nature may have originated from the stable A1 or A2 type through sexual reproduction, aging (19), or exposure to various fungicides (1,4, 19). The fact that the mating type of the A1 and the A2 isolates of *P. infestans* can be changed to the opposite type and that the self-fertile type can generate both the A1 and the A2 mating type offspring suggests that some members of the A1 and A2 mating types existing in nature may have originated from the opposite type or the self-fertile type before or after arriving at the present host countries.

The composition of *P. infestans* in each country outside Mexico may include direct asexual descendants of pioneers consisting of the A1 and/or A2 mating type and offspring of pioneer descendants whose sex had been changed at various asexual generations through natural courses such as aging and sexual reproduction or exposure to environmental stresses such as toxic chemicals. The composition may also include newcomers that may be the A1 and/or A2 mating type. All isolates of *P. infestans* recovered outside Mexico, both A1 and A2, thus require rigorous testing before their origins can be ascertained.

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